Chapter

Applications of Biological Surface Active Compounds in Remediation Technologies

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Abstract

Many microorganisms synthesize a wide range of surface active compounds (SACs), classified according to their molecular weights, properties and localizations. The low molecular weight SACs or biosurfactants lower the surface tension at the air/water interfaces and the interfacial tension at oil/water interfaces, whereas the high molecular weight SACs, also known as bioemulsifiers, are more effective in stabilizing oil-in-water emulsions. The ability to biosynthesize SACs is, often, coupled with the ability of these microorganisms to grow on immiscible carbon sources, such as hydrocarbons. Different mechanisms are involved in the SACs interactions between microbial cells and immiscible hydrocarbons including: (i) emulsification, (ii) micellarization, (iii) adhesion-deadhesion of microorganisms to and from hydrocarbons and (iv) desorption of contaminants. These naturally occurring phenomena can be exploited by adding bioemulsifiers and biosurfactants into environments where bioremediation/biodegradation rates of organic pollutants is to be enhanced. However, analysis of the current literature show some cases where the complex interactions among SACs, microbial cells, organic substrates and environmental media led to an inhibition of the biodegradation. The understanding of the different physiological roles of SACs in microbial communities is fundamental in order to develop more effective remediation technologies exploiting both synthetic surfactants and microbial SACs. The physio-chemical properties of some microbial SACs have been exploited in hydrocarbon-contaminated soils washing and in mobilisation of soil-bound metal in metal-contaminated soils. Our ability to analyse the microbial diversity in the natural environments will expand our knowledge on microbial SACs with respect to their exploitation for commercial applications and their roles in the physiology of the producing microorganisms.

Microbial Surface Active Compounds

Structures and Properties

Many prokaryotic and eukaryotic microorganisms synthesize a wide range of structurally different amphiphilic molecules containing both hydrophilic and hydrophobic (typically a hydrocarbon) moieties. The structural features of amphiphiles confer them the ability to concentrate and alter the conditions at interfaces. Interface is a term describing a surface which forms a boundary between two different phases, such as gas/liquid, two immiscible liquids, solid/liquid. Due to their superficial properties, amphiphilic microbial metabolites have been usually referred to as Surface

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Active Compounds (SACs). Neu[1] divided SACs into three different classes: (i) biosurfactants are defined as low molecular weight SACs (e.g., glycolipids, lipopeptides); (ii) amphiphilic polymers are defined as high molecular weight SACs with a hydrophobic region at one end of the molecule (e.g., lipopolysaccharides, lipoteicoic acids); (iii) polyphilic polymers are defined as high molecular weight SACs with hydrophobic groups distributed across the entire polymeric molecule (e.g., hydrophobic polysaccharides, emulsan). The low molecular weight SACs or biosurfactants lower the surface tension at the air/water interfaces and the interfacial tension at oil/water interfaces, whereas the high molecular weight SACs, also called bioemulsifiers, are more effective in stabilizing oil-in-water emulsions.[2]

Comparing the properties of different biosurfactants, surface and interfacial tensions are parameters used as a measure of biosurfactant effectiveness. When a biosurfactant is added to air/water or oil/water systems at increasing concentrations, a reduction of the surface tension is observed up to a critical level, above which the amphiphilic molecules associate readily to form supramolecular structures, such as micelles, bilayers and vesicles.[3] The concentration at which surfactants begin to form micelles is known as the critical micelle concentration (CMC) which is used to evaluate biosurfactant efficiency.

In a heterogeneous system, an emulsion is the mixture of two immiscible liquids which is formed when one liquid phase is dispersed as microscopic droplets in an other continuous phase.[3] The activity of different bioemulsifiers is compared by assaying their ability to stabilize a water/oil emulsion or generate turbidity due to suspended hydrocarbons in an aqueous system.[4,5]

The best studied low molecular weight SACs so far are glycolipids and lipopeptides.[2] Glycolipids are disaccharides acylated with long chain fatty acids or hydroxyl fatty acids. Among them, the best-characterized structural subclasses are rhamnolipids produced by several *Pseudomonas* species, sophorolipids synthesized by different species of the yeast *Candida* (formerly *Torulopsis*) and trehalolipids found in *Rhodococcus* and other actinomycetes.[6,7] Most of the biosurfactants produced by rhodococci are trehalose mycolates consisting of a trehalose residue linked by an ester bond to mycolic acids, long α-alkyl β-hydroxy fatty acids.[8] Lipopeptides are low molecular weight SACs showing potent surface activities. A variety of structurally different variants is produced by several *Bacillus* species. *Bacillus subtilis* produces a cyclic lipopeptide called surfactin or subtilisin which has been reported as the most active biosurfactant discovered todate.[9]

High molecular weight SACs are produced by a wide diversity of Bacteria (Gram-positive and Gram-negative) and Archaea. Most of the emulsifiers are composted by mixtures of hydrophobic and hydrophilic polymers. The most extensively studied bioemulsifiers are the ones produced by different *Acinetobacter* species.[2] An example of well-characterized high molecular weight SAC is Emulsan, an effective emulsifier produced by the *Acinetobacter lwoffii* strain RAG-1 (formerly *Acinetobacter calcoaceticus*). Emulsan is a complex mixture of an anionic heteropolysaccharide and proteins. It presents a polyphilic structure being composed of fatty acids attached, over the entire molecule, to the polysaccharidic backbone. Its emulsification activity is due to the tight affinity of emulsan for oil/water interfaces. Emulsan has been found to exibit high specificity: it is not able to emulsify pure aliphatic, aromatic, or cyclic hydrocarbons but it efficiently emulsifies mixtures containing the appropriate proportions of aliphatic and aromatic (or cyclic) alkanes.[2]

Novel Microbial Surface Active Compounds

Most research on microbial SAC has been confined, mostly, to few well-characterized molecules produced by a small number of microbial genera (*Pseudomonas, Candida, Bacillus, Acinetobacter*). Consequently, our understanding of the diversity, physiological roles and potential applications of microbial SACs is limited to a relatively narrow spectrum of microbial metabolites and biological systems. Only few studies were concerned with the phylogenetic diversity of SAC-producing microorganisms and the majority of the producing microorganisms has been isolated from a narrow range of environments, mainly undisturbed and hydrocarbon contaminated soils or heavy metal contaminated soils.[10-13]

In the last few years, a growing number of new SAC-producing microorganisms have been described although their products often remain uncharacterized in respect to their chemical structures. Bodour et al[14] reported a new glycolipid class, the flavolipids, produced by a *Flavobacterium* strain isolated from soil. Flavolipids exhibit a unique polar moiety which features citric acid and two cadaverine molecules and display strong surfactant and emulsifying activities. The cold-adapted *Halomonas* sp. strain ANT-3b, isolated from Antarctic seawater, has been also recently reported to produce a new high molecular weight glycolipidic bioemulsifier.[15] Bonilla et al[16] also reported the production of an exopolysaccharide with emulsifying activity by a *Pseudomonas* strain which has a significantly different chemical composition to previous reports.

The Roles of SACs in Hydrocarbon Metabolism

Microbial ability to biosynthesize SACs is, often, coupled with their ability to grow on immiscible carbon sources although many produce amphiphilic metabolites from miscible carbon sources.[17] SACs can be intracellular, cell surface bound or extracellular compounds.[1] The kinetics of SAC production differ among various biological systems[3] and are produced by a variety of microorganisms in heterogeneous growth conditions leading to varying roles in the physiology of the producing microorganisms.[9] The physiological roles proposed for microbial SACs have been recently reviewed by Van Hamme et al.[18] SACs appear to play a role in different behaviours which microbial cells carry out when they contact interfaces. Among the roles proposed for microbial SACs are motility (gliding, swarming, de-adhesion from surfaces), cell-cell interactions (biofilm formation, maintenance and maturation, quorum sensing, amensalism, pathogenicity), cellular differentiation, substrate accession as well as avoidance of toxics elements and compounds.

In this chapter, we examine the proposed roles for SACs with respect to the interactions between microbes and hydrocarbons. Particularly, we discuss the different strategies evolved by microorganisms to overcome the low solubility of hydrocarbons, access to hydrocarbons before transportation into cells and adhesion-deadhesion of microbial cells from and to hydrocarbon surfaces.[19,20] Understanding of the different physiological roles of SACs in microbial communities is fundamental in order to develop more effective remediation technologies exploiting both synthetic surfactants and microbial SACs and techniques useful in evaluating the impact of treatments on microbial communities and outcomes of remediation processes.

Microbial Access to Hydrocarbons

Hydrocarbon metabolism is always restricted to water/hydrocarbon interfaces since the oxygenases involved in their catabolic pathways are never extracellular but always membrane-bound enzymes.[19] Thus, microbial growth on hydrocarbons can be limited by the interfacial surfaces leading to a linear growth rather than exponential one. Extracellular biosurfactants and bioemulsifiers increase oil/water interfaces enhancing substrate mass transfer and allowing more microorganisms to contact the hydrocarbon substrates. Emulsifiers increase the hydrocarbon/water interfaces stabilizing oil droplets in the water/oil emulsion. On the other hand, when a surfactant is present in an oil/water system at concentrations above its CMC, the oil solubility, dramatically, increases due to the aggregation of surfactant micelles. The hydrophobic moieties of the surfactant molecules cluster together exposing the hydrophilic ends to the aqueous phase on the exterior. Consequently, the core of micelles becomes a compatible environment for hydrophobic organic molecules. The process is known as pseudosolubilization.[21]

The ability of different microorganisms to access hydrocarbons depends on their cell surface hydrophobicity. High cell-hydrophobicity allows them to directly contact oil drops and solid hydrocarbons while low cell hydrophobicity permits their adhesion to micelles or emulsified oils.[19,20] Three different mechanisms of cell access to hydrocarbons have been postulated: (i) access to water-solubilize hydrocarbons, (ii) direct contact of cells with large oil drops, (iii) contact with pseudosolubilized or emulsified oil. The first mechanism is limited to low molecular weight hydrocarbons since the hydrocarbon solubility, dramatically, decreases with increased molecular weights. In rhodococci, cells are hydrophobic due to the presence of a hydrophobic mycolic acid

layer in their cell walls and the major hydrocarbon accession mode is likely to be direct contact of hydrophobic cells with large oil drops (Fig. 1a).[8,21] *Rhodococcus* genus belongs to mycolic acid-containing actinomycetes including also *Gordonia*, *Nocardia*, *Corynebacterium*, *Tsukamurella* and *Mycobacterium* genera. In *Rhodoccoccus* spp., mycolic acids are found attached to the cell wall arabinogalactans and partially free in the form of trehalose mycolates. Arabinogalactan-bound mycolic acids, as well as free trehalose mycolates, are thought to be localized in the outer layer of the cell wall, where they form the basis of an outer lipid permeability barrier.[22] Thus, the cell-associated amphiphilic trehalose mycolates seems to play a structural role in the rhodococci cell wall. On the other hand, the access to hydrocarbons in *Pseudomonas* strains relays on the release in the culture broths of the extracellular surfactants, rhanmolipids, which enhance the hydrocarbon apparent solubility. The hydrophilic surface allows *Pseudomonas* cells to interact with the hydrophilic outer layer of the hydrocarbon-containing micelles (Fig. 1b).[23]

SACs are thought to play a role in regulating the cell surface hydrophobicity thereby controlling adhesion-deadhesion of microbial cells to and from hydrocarbon surfaces.[1,9,24] Microorganisms either increase or decrease their cell hydrophobicity by respectively exposing outwardly or inwardly the hydrophobic moieties of the cell-bound SACs. For example, the cell-surface hydrophobicity of *A. lwoffii* RAG-1 is reduced by the presence of emulsan, a cell-bound bioemulsifier.[2,25] During the exponential phase of growth on oil mixtures, RAG-1 cells are attached to the oil droplets and emulsan is cell-bound in the form of a minicapsule. After bacteria have consumed long chain *n*-alkanes in the oil droplets, RAG-1 cells become starved being unable to metabolize any of the other oil components which leads to the release of emulsan minicapsule from the cell surfaces desorbing starved cells from hydrocarbons and forming a polymeric film on the *n*-alkane-depleted oil droplets. This hydrophilic film layer is laid over the exhausted droplets to which RAG-1 cells cannot attach anymore therefore compelling them to attach to fresh oil droplets.[2]

Altering Access Mode

Franzetti et al[24] recently suggested that some microbial SACs play a role in changing the substrate access mode during the different growth stages on hydrocarbons. They observed that *Gordonia* sp. strain BS29 grown on hydrocarbons synthesizes both cell-bound glycolipid biosurfactants and extracellular bioemulsifiers. During early exponential phase of growth on *n*-hexadecane, BS29 surface is hydrophobic and cells access large oil drops through direct contact (Fig. 1a). During the late exponential phase, the cell surface becomes hydrophilic. This change in surface hydrophobicity may be due to cell-bound SACs which expose their hydrophilic moieties toward the water phase masking the highly hydrophobic character of the mycolic acid layer. Consequentially, the hydrophilic surface allows cells to attach to the hydrophilic outer layer of the emulsified oil droplets (Fig. 1c). Ron and Rosenberg[9] have suggested that there are conceptual difficulties in understanding the evolutionary advantages of producing extracellular bioemulsifiers, since it is impossible to obtain an oil emulsion available only for the producing strain in an open system. However, the population-specific interaction between BS29 and microemulsion (mediated by the regulation of cell hydrophobicity and emulsifier biosynthesis) could allow BS29 to take advantage of the emulsion over the other microbial populations.

Remediation Technologies

SACs have recently been evaluated in bench and field-scale experimentations as substitutes for chemically synthesized surfactants to improve rate of contaminant removal in soil and water remediation processes. Microbial SACs find potential applications within physicochemical technologies for remediation of both organic and metal contaminations, such as in situ soil flushing and ex situ soil washing for remediation of unsaturated zone , pump and treat for aquifer remediation,[26-28] and also in bioremediation technologies to improve the biodegradation rate of organic compounds.[28] A wide range of other different potential commercial exploitations have been described not only for oil industry, such as microbial enhanced oil recovery, oil transportation and tank cleaning, but also in medicine, cosmetics and food industries.[2,29,30]

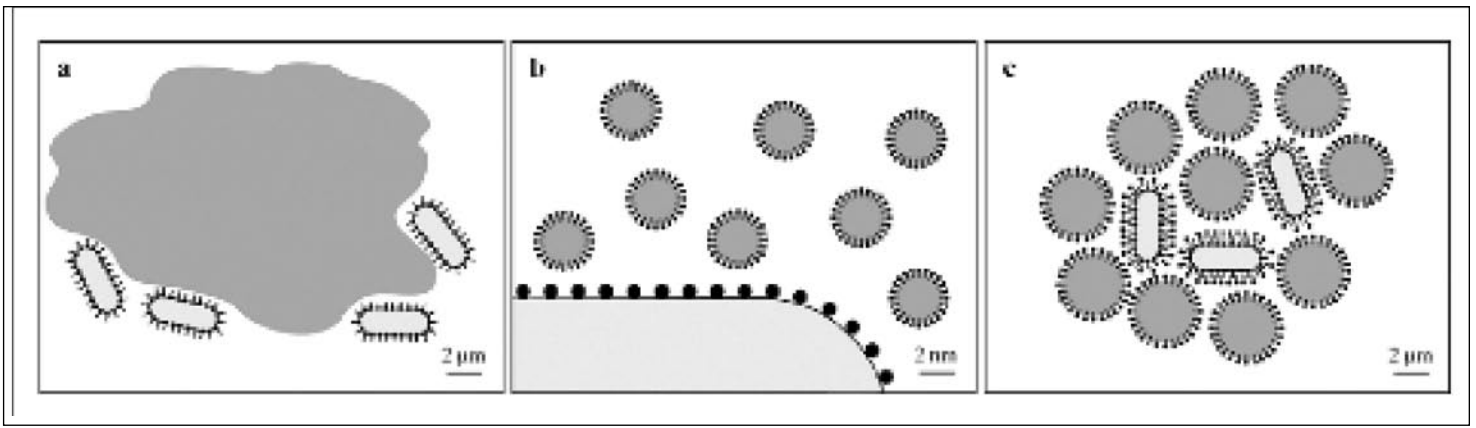

Figure 1. Different hydrocarbon accession modes in microorganisms: direct contact of cells with large oil drops (a), contact with pseudosolubilized oil (b) and emulsified oil (c). Dots and lines represent hydrophilic and hydrophobic moieties of microbial SACs, respectively (not on scale).

Bioremediation

Physicochemical properties of SACs are involved in the interaction between microbial cells and immiscible hydrocarbons by the following mechanisms[9,31]: (i) emulsification, (ii) micellarization, (iii) adhesion-deadhesion of microorganisms to and from hydrocarbons and (iv) desorption of contaminants. These naturally occurring phenomena can be exploited to enhance bioremediation treatments by adding biological SACs (Table 1) and chemical surfactants (Table 2).

Emulsification

Despite their potentials, microbial emulsifiers have been rarely evaluated as enhancers of hydrocarbon biodegradation in bioremediation. Barkay et al[32] showed that Alasan, produced by *Acinetobacter radioresistens* more than doubled the rate of [^{14}C] fluoranthene mineralization and significantly increased the rate of [^{14}C] phenanthrene mineralization by *Sphingomonas paucimobilis* EPA505.

Micellarization

When a surfactant is present at concentrations above its CMC, a significant fraction of the hydrophobic contaminants partitioned in the surfactant micelle cores. This, generally, results in an increase in the bioavailability of the hydrophobic contaminants to the degrading-microorganisms thus enhancing their biodegradation rate.[31] Several researchers demonstrated that rhamnolipid addition to contaminated soils above CMC both accelerated the biodegradation of hexadecane, octadecane, *n*-paraffins, creosotes and other hydrocarbon mixtures and enhanced the bioremediation of petroleum sludges.[33-36] Furthermore, the addition of glycolipids improved the biodegradation of chlorinated hydrocarbons.[37] Pesticide biodegradation was also reported to be promoted by surfactin.[38]

On the other hand, other studies showed the organic contaminants trapped into micelle cores become less bioavailable to the microorganisms resulting in an inhibition of their degradation. Witconol SN70, a nonionic alcohol ethoxylate surface active compound, inhibited the mineralization of hexadecane and phenanthrene.[39] Doses of four surfactants (Tween 20, sodium dodecyl sulfonate, tetradecyl trimethyl ammonium bromide, Citrikleen) at ≥ CMCs, inhibited mineralization of phenanthrene in a soil-water system.[40] In aqueous media, the biodegradation of four PCB congeners by *Pseudomonas* LB-400 was inhibited by Igepal CO-630, a nonionic surfactant, at concentrations above its CMC.[41] Also other cases of inhibition of biodegradation due the addition of surfactants have been observed and believed to be due to the surfactants providing a more easily degradable carbon source alternative to the contaminants.[42,43]

Regulation of Adhesion-Deadhesion of Microorganisms to Hydrocarbons

A proposed role for microbial SACs is the regulation of the adhesion-deadhesion of microorganisms to and from hydrocarbons. The exploitation of this natural roles consists in the addition of surfactants to increase the hydrophobicity of degrading microorganisms which allows cells to access to hydrophobic substrates more easily.[44,45] Al-Tahhan et al[46] demonstrated that sub-CMC levels of rhamnolipids caused the release of LPS by *Pseudomonas* spp., a phenomenon that rendered the cell surface more hydrophobic allowing a more efficient uptake of hexadecane. Normann et al[35] demonstrated that rhamnolipid by *P. aeruginosa* UG2 stimulated the degradation of hexadecane by the same organism facilitating the hydrocarbon uptake. This rhamnolipid did not stimulate to the same extent the biodegradation of hexadecane by four other strains (*A. lwoffii* RAG1, *Rhodococcus erythropolis* DSM 43066, *R. erythropolis* ATCC 19558 and strain BCG112), nor was degradation of hexadecane stimulated by addition of their own biosurfactants. More recently, Zhong et al[47] studied the adsorption of dirhamnolipid biosurfactants on cells of *P. aeruginosa*, *B. subtilis* and *Candida lipolytica*. Their results showed that the adsorption was specific to the microorganisms and depended on the physiological status of their cells. Furthermore, biosurfactant adsorption caused the cell surface hydrophobicity to change depending on both the rhamnolipid concentrations and the cell physiological conditions.

Table 1. Effect of microbial SACs on biodegradation of organic compounds

Producing Microorganisms	SACs (E/S)[1]	Experimental Systems	Degrading Microorganisms	Pollutants	Effect on Degradation[2]	Mechanisms of Degradation Enhancement/Inhibition	Ref
Acinetobacter radioresitant KA53	Alasan (E)	Liquid cultures	*Sphingomonas paucimobilis* EPA 505	Phenanthrene	E	Increasing phenanthrene solubility	32
Acinetobacter calcoaceticus RAG1	Emulsan (E)	Liquid cultures	*Acinetobacter calcoaceticus* RAG1, *Rhodococcus erythropolis* DSM 43066, *R. erythropolis* ATCC 19558, *Pseudomonas aeruginosa* UG2	Hexadecane	I	Altering accession to hydrocarbon	35
Pseudomonas aeruginosa	Rhamnolipid (S)	Liquid cultures	*Pseudomonas aeruginosa*	Hexadecane	E	Involvement in hexadecane uptake	33
Pseudomonas aeruginosa UG2	Rhamnolipid (S)	Liquid cultures	*Pseudomonas aeruginosa* UG2, PG201 and ATCC 15528	Hexadecane	E	Facilitating the hydrocarbon uptake	35
Pseudomonas aeruginosa UG2	Rhamnolipid (S)	Liquid cultures	*Rhodococcus erythropolis* DSM 43066, *R. erythropolis* ATCC 19558,	Hexadecane	I	Altering accession to hydrocarbon	35
Pseudomonas aeruginosa	Rhamnolipid (S)	Soil microcosms	Soil autochthonous community/ degrading consortium	Petroleum sludge	E	Increasing bioavailability	36
Pseudomonas aeruginosa	Rhamnolipid (S)	Liquid cultures	*Streptomyces* PS1/5	Trifluralin,	E	Increasing bioavailability	37
Pseudomonas aeruginosa	Rhamnolipid (S)	Liquid cultures	*Streptomyces* PS1/5	Atrazine	I	Providing alternative substrate	37
Pseudomonas aeruginosa	Rhamnolipid (S)	Liquid cultures	Degrading consortia from a contaminated cattle dip	Coumaphos	E	Increasing bioavailability	37

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Table 1. Continued

Producing Microorganisms	SACs (E/S)[1]	Experimental Systems	Degrading Microorganisms	Pollutants	Effect on Degradation[2]	Mechanisms of Degradation Enhancement/Inhibition	Ref
Pseudomonas aeruginosa	Rhamnolipid (S)	Soil slurry	*Streptomyces* PS1/5	Trifluralin, Atrazine	NA	-	37
Pseudomonas aeruginosa	Rhamnolipid (S)	Soil slurry	Degrading consortia from a contaminated cattle dip	Coumaphos	E	Increasing bioavailability	37
Pseudomonas aeruginosa ATCC 9027	Rhamnolipid (S)	Liquid cultures	Pseudomonas *aeruginosa* ATCC 9027/ATCC 27853	Hexadecane	E	Increasing cell hydrophobicity	46
Pseudomonas aeruginosa sp.	Rhamnolipid (S)	Liquid cultures	*Pseudomonas aeruginosa*	Hexadecane	E	Increasing cell hydrophobicity/ pseudosolubilisation	44
Pseudomonas aeruginosa sp.	Rhamnolipid (S)	Soil column	Soil autochthonous community	Phenanthrene	E	Desorption of phenatrene from soil	50
Pseudomonas aeruginosa UG2	Rhamnolipid (S)	Silica column	*Pseudomonas aeruginosa* UG2	Hexadecane	E	Desorption of hexadecane from micropores	54
Bacillus subtilis MTCC1427	Surfactin	Liquid cultures/soil: water slurry	Consortium of two bacterial cultures	Endosulfan	E	Increasing bioavailability	38

[1]SACs; E: emulsifier; S: surfactant; [2]Effect on degradation: E: Enhancement; I: Inhibition; NA: not affected.

Table 2. Effect of chemical synthesized surfactants on biodegradation of organic compounds

Surfactant	Experimental System	Degrading MOs	Pollutants	Effect on Degradation[1]	Mechanisms of Degradation Enhancement/Inhibition	Ref
Triton X-100	Liquid culture	*Streptomyces* PS1/5	Trifluralin and Atrazine	I	Lowering bioavailability	37
Triton X-100	Soil slurry	*Streptomyces* PS1/5	Trifluralin and Atrazine	NA	-	37
Triton X-100	Soil slurry	degrading consortia from a contaminated cattle dip	Coumaphos	I	Lowering bioavailability	37
Triton X-100	Liquid cultures	*Mycobacterium* sp.; *Pseudomonas* sp	Anthracene	I	Decreasing cell hydrophobicity—inhibition cell-substrate access	48
Triton X-100	Soil	Soil autochthonous community	Phenanthrene	E	Desorption of phenatrene from soil	53
Triton X-100; PLE10	Liquid cultures	*Pseudomonas* sp. 8909N	Naphthalene	E	Increasing of dissolution rate	51
Brij 56	Liquid cultures	Gram positive bacterium	Diesel fuel	NA	-	43
Tween 80	Liquid cultures	Gram positive bacterium	Diesel fuel	E	Pseudosolubilisation	43
Tween 80; Brij 56	Soil slurry	Soil autochthonous community	Diesel fuel	I	Co-degradation and soil sorption (Tween 80); toxicity and lowering bioavailability (Brij 56)	43
Witconol SN70	Soil	Soil autochthonous community	Hexadecane, phenathrene	I	Toxicity/codegradation	39
Tween 20, sodium dodecyl sulfonate, tetradecyl trimethyl ammonium bromide, citrikleen	Liquid cultures/soil water slurry	Degrading consortium	Phenanthrene	I	Toxicity of surfactant and solubilized phenanthrene	40

[1]Effect on degradation; E: Enhancement; I: Inhibition; NA: not affected.

Cases of inhibition of microbial degradation due to surfactant-induced change in surface hydrophobicity have also been reported. Chen et al[48] observed that low concentration (0.09 CMA) of Triton X-100 inhibited the growth on solid anthracene of a *Mycobacterium* sp. strain and a *Pseudomonas* sp. strain. The causes of inhibition were believed to be the sorption of the surfactant onto both microbial cell surfaces and anthracene particles.

Desorption of Contaminants

Organic compounds can often strongly bind to particles on porous materials, such as soils therefore, becoming trapped into micropores. This, usually, does not allow rapid remediation and can lead to extended remediation periods. Several studies have shown that the mass transfer from ab/adsorbed phase to liquid is the controlling mechanism of biodegradation rate.[49] In these cases, biosurfactants can enhance the bioavailability of contaminants even at concentrations below the CMC.[28] Phenomena associated with this mechanism include a reduction of surface and interfacial tensions, capillary force and wettability and an increase of contact angle. At concentrations below CMC, surfactants reduce the surface and interfacial tensions between air/water, oil/water and soil/water systems. In a soil/oil system, surfactants increase the contact angle and reduce the capillary force holding together oil and soil particles due to the reduction of the interfacial force. Surfactants have been used to stimulate the dissolution of non-aqueous phase liquids initially present in soils,[50] the dissolution of solid contaminants[51] and the desorption and transport of soil-sorbed contaminants.[52,53]

Noordman et al[54] investigated the effect of the rhamnolipid biosurfactant on hexadecane degradation in the case of substrate entrapped in small soil pore sizes (6 nm). Even in low mixing conditions, rhamnolipids stimulated the release of entrapped substrates and enhanced uptake by cells.

Soil Washing

Hydrocarbon Contaminated Soils

The prospects of using biosurfactants in hydrocarbon-contaminated soil washing depend on the capacity of these compounds to enhance the desorption and dissolution of the polluting organic compounds and increase the rate of transport of contaminants in soils. The mechanisms involved in the hydrocarbon removal from soils are related to the mechanisms involved in increasing bioavailability for bioremediation purposes. The properties of stabilizing oil/water emulsions and increasing hydrocarbon solubility may enhance both the biodegradation rate and the hydrocarbon removal rate from soils.[55] These mobilization and solubilization effects occur at both concentration below and above the CMC. The application of microbial SACs to remove contaminants from soils is a technology characterized by some minor degree of uncertainty than the SAC-enhanced bioremediation, since only the chemicophysical properties of the biosurfactants and not their effects on cell surface properties and microbial metabolisms drive the removal efficiency.

The use of chemical surfactants has been reported to be efficient in removing hydrocarbons from soils. Lee at al.[56] reported that non ionic surfactants removed more than 80% of total hydrocarbons from soils. Billingsley et al[41] demonstrated interesting differences in the effects of non-ionic and anionic surfactants on the removal and bioavailability of PCBs. Nonionic surfactants washed more PCBs from soils while the substrate into anionic surfactants micelle cores were more available for biodegradation by a PCB-degrading *Pseudomonas* sp. Microbial SACs often exhibited better capacity of removing hydrocarbons than their synthetic counterparts. The more commonly studied biosurfactants, such as rhamnolipids and surfactin, have been successfully evaluated in washing of soils contaminated by crude oils, PAHs and chlorinated hydrocarbons.[28] In several cases, the removal efficiency was very high (up to 80%) and depended on both the contact time and biosurfactant concentration.[50,57] Rhamnolipids have been reported to release three times as much oil as water alone from the beaches in Alaska after the Exxon Valdez tanker spill.[58] Van Dyke et al[59] have reported that rhamnolipids, at a concentration of 5 g/l, could remove approximately 10% more hydrocarbons from a sandy loam soil than sodium dodecyl sulfate. Biosurfactants appeared to be more effective in increasing the apparent solubility of PAHs by up to five times as compared to

chemical surfactants.[60,61] Biosurfactants have also found applications in aquifer remediation due to their ability to reduced interfacial tension between dense the non-aqueous phase liquids and groundwaters.[62,63]

Metal Contaminated Soils

The interactions between surfactants and metals are not fully understood. It is known that surfactants can remove metals from surfaces by different mechanisms. Non ionic metals can form complexes with biosurfactants, enhancing their removal from porous media.[64] Anionic surfactants interact with cationic metals leading to their desorption from surfaces.[27] Nevertheless, also cationic surfactants can play a role by competitive binding to negative charged binding sites. The first studies on biosurfactant-metal complex were carried out by Tan et al[65] They demonstrated the rapid formation of monorhamnolipid-metal complex. Rhamnolipids have been evaluated for their affinity to metal cations.[66] $K^+ < Mg^{2+} < Mn^{2+} < Ni^{2+} < Co^{2+} < Ca^{2+} < Hg^{2+} < Fe^{3+} < Zn^{2+} < Cd^{2+} < Pb^{2+} < Cu^{2+} < Al^{3+}$ are the cations in order (from lowest to highest) of affinity with rhamnolipids. Mulligan and coworkers extensively studied the potential of rhamnolipids, sophorolipids and surfactin in washing of metal-contaminated soils and sediments.[26] Mulligan and Young[67] studied the effect of biosurfactants by *Pseudomonas* sp., *Bacillus* sp. and *Candida* sp. on zinc and copper removal from soils and demonstrated that anionic surfactants are able to selectively remove metals oxide, carbonate and organic fraction from soils. Rhamnolipids successfully removed heavy metals from an oil cocontaminated soil[68] and heavy metal contaminated sediments.[26] Batch soil washing experiments were carried out to evaluate the feasibility of using surfactin for the removal of heavy metals from contaminated soils and sediments. By a series of five soil washings, removals of 70% and 22% of copper and zinc, respectively were reported.[26] Surfactin was able to remove the metals by both sorption at the soil particle interphase and metal complexation.

Future applications of bioemulsifiers in remediation of heavy metals and radionuclides can be now envisaged. Several microbial polysaccharides have been shown to bind heavy metals. Emulsan by *A. lwoffii* RAG-1 forms stable oil-in-water emulsions. In this system, metal ions bind primarily at the oil/water interphase enabling their recovery and concentration from relatively dilute solutions. Cations bound to the emulsion can be completely removed to the water phase when pH was lowered.[69]

Conclusions and Prospects

The heterogeneity of SAC structural types and properties results in a broad spectrum of potential applications in environmental remediation as well as in the oil industry, agriculture, medicine, cosmetic and food industries.[29] Our increasing ability to analyze the microbial diversity in natural environments is expected to expand our knowledge on microbial SACs with respect to their exploitation for commercial applications and their roles in the physiology of the producing microorganisms. During the past few years, high throughput methods have been generated for the systematic screening of SAC-producing microorganisms.[70,71] Unfortunately, only a small percentage of microorganisms can be cultivated from environmental samples using traditional cultivation techniques.[72] In order to overcome the problems associated with cultivation of microorganisms, new cultivation methods have been developed in order to increase the number of culturable bacterial species and investigate the previously inaccessible resources that these microorganisms potentially have.[73]

References

1. Neu T. Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiol Rev 1996; 60:151-166.
2. Rosenberg E, Ron EZ. High- and low-molecular-mass microbial surfactants. Appl Microbial Biotechnol 1999; 52:154-162.
3. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev 1997; 61:47-64.
4. Cooper DG, Goldenberg BG. Surface-active agents from two Bacillus species. App Environ Microbiol 1987; 53:224-229.

5. Rosenberg E, Zuckerberg A, Rubinovitz C et al. Emulsifier of Arthrobacter RAG-1: isolation and emulsifying properties. Appl Environ Microbiol 1979; 37:402-408.
6. Ito S, Inoue S. Sophorolipids from Torulopsis bombicola: possible relation to alkane uptake. Appl Environ Microbiol 1982; 43:1278-1283.
7. Kretschmer A, Bock H, Wagner F. Chemical and physical characterization of interfacial-active lipids from Rhodococcus erythropolis grown on n-alkanes. Appl Environ Microbiol 1982; 44:864-870.
8. Lang S, Philp JC. Surface-active lipids in rhodococci. Anton Leeuw Int J G 1998; 74:59-70.
9. Ron EZ, Rosenberg E. Natural roles of biosurfactants. Environ Microbiol 2001; 3:229-236.
10. Bodour AA, Drees KP, Maier RM. Distribution of biosurfactant-producing bacteria in undisturbed and contaminated arid southwestern soils. Appl Environ Microbiol 2003; 6:3280-3287.
11. Bento FM, Camargo FA, Okeke BC et al. Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. Microbiol Res 2005; 160:249-255.
12. Batista SB, Mounteer AH, Amorim FR et al. Isolation and characterization of biosurfactant/bioemulsifier-producing bacteria from petroleum contaminated sites. Bioresour Technol 2006; 97:868-875.
13. Ruggeri C, Franzetti A, Bestetti G et al. Isolation and screening of surface active compound-producing bacteria on renewable substrates. Proceedings of second international conference on environmental, industrial and applied microbiology. Seville, Spain. In press.
14. Bodour AA, Guerrero-Barajas C, Jiorle BV et al. Structure and characterization of flavolipids, a novel class of biosurfactants produced by Flavobacterium sp. Strain MTN11. Appl Environ Microbiol 2004; 70:114-120.
15. Pepi M, Cesàro A, Liut G et al. An antarctic psychrotrophic bacterium Halomonas sp. ANT-3b, growing on n-hexadecane, produces a new emulsyfying glycolipid. FEMS Microbiol Ecol 2005; 53:157-166.
16. Bonilla M, Olivaro C, Corona M et al. Production and characterization of a new bioemulsifier from Pseudomonas putida ML2. J Appl Microbiol 2005; 98:456-463.
17. Makkar RS, Cameotra SS. An update on the use of unconventional substrates for biosurfactant production and their new application. Appl Microbiol Biotechnol 2002; 58:428-434.
18. Van Hamme JD, Singh A, Ward OP. Physiological aspects. Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol Adv 2006; 24:604-620.
19. Van Hamme JD, Singh A, Ward OP. Recent advances in petroleum microbiology. Microbiol Mol Biol R 2003; 6:503-549.
20. Bouchez-Naitali M, Rakatozafy H, Marchal R et al. Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake. J Appl Microbiol 1999; 8:421-428.
21. Urum K, Pekdemir T. Evaluation of biosurfactants for crude oil contaminated soil washing. Chemosphere 2004; 57:1139-1150.
22. Sutcliff IC. Cell envelope composition and organisation in the genus Rhodococcus. Anton Leeuw Int J G 1998; 74:49-58.
23. Van Hamme JD, Ward OP. Physical and metabolic interactions of Pseudomonas sp. Strain JA5-B45 and Rhodococcus sp. Strain F9-D79 during growth on crude oil and effect of a chemical surfactant on them. Appl Environ Microbiol 2001; 67:4874-4879.
24. Franzetti A, Bestetti G, Caredda P et al. Surface-active compounds and their role in bacterial access to hydrocarbons in Gordonia strains. FEMS Microbiol Ecol 2008; 63:238-248.
25. Rosenberg E, Gottlieb A, Rosenberg M. Inhibition of bacterial adherence to hydrocarbons and epithelial cells by emulsan. Infect Immun 1983; 39:1024-1028.
26. Mulligan CN, Yong RN, Gibbs BF. An evaluation of technologies for the heavy metal remediation of dredged sediments. J Hazard Mater 2001; 85:145-163.
27. Christofi N, Ivshina IB. Microbial surfactants and their use in field studies of soil remediation. J Appl Microbiol 2002; 93:915-929.
28. Mulligan CN. Environmental applications for biosurfactants. Environ Pollut 2005; 133:183-198.
29. Banat IM, Makkar RS, Cameotra SS. Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 2000; 53:495-508.
30. Kourkoutas Y, Banat IM. Biosurfactant production and application. In: Pandey AP, ed. The Concise Encyclopedia of Bioresource Technology. Philadelphia: Haworth Reference Press, 2004:505-515.
31. Singh A, Van Hamme JD, Ward OP. Surfactants in microbiology and biotechnology: Part 2. Application aspects. Biotechnol Adv 2007; 25:99-121.
32. Barkay T, Navon-Venezia S, Ron EZ et al. Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bioemulsifier Alasan. Appl Environ Microbiol 1999; 65:2697-2702.
33. Beal R, Betts WB. Role of rhamnolipid biosurfactants in the uptake and mineralization of hexadecane in Pseudomonas aeruginosa. J Appl Microbiol 2000; 89:158-68.
34. Maier RM, Soberón-Chávez G. Pseudomonas aeruginosa rhamnolipids: biosynthesis and potential applications. Appl Microbiol Biotechnol 2000; 54:625-633.

35. Noordman WH, Janssen DB. Rhamnolipid stimulates uptake of hydrophobic compounds by Pseudomonas aeruginosa. Appl Environ Microbiol 2002; 68:4502-4508.
36. Rahman KSM, Rahman, TJ, Kourkoutoas Y et al. Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioresour Technol 2003; 90:159-168.
37. Mata-Sandoval JC, Karns J, Torrents A. Influence of rhamnolipids and triton X-100 on the biodegradation of three pesticides in aqueous phase and soil slurries. J Agric Food Chem 2001; 49:3296-3303.
38. Awasthi N, Kumar A, Makkar R et al. Enhanced biodegradation of endosulfan, a chlorinated pesticide in presence of a biosurfactant. J Environ Sci Health 1999; B34:793-803.
39. Colores GM, Macur RE, Ward DM et al. Molecular analysis of surfactant-driven microbial population shifts in hydrocarbon-contaminated soil. Appl Environ Microbiol 2000; 66:2959-2964.
40. Bramwell DP, Laha S. Effects of surfactant addition on the biomineralization and microbial toxicity of phenanthrene. Biodegradation 2000; 11:263-277.
41. Billingsley KA, Backus SM, Ward OP. Effect of surfactant solubilization on biodegradation of polychlorinated biphenyl congeners by Pseudomonas LB400. Appl Microbiol Biotechnol 1999; 52:255-260.
42. Goudar C, Strevett K, Grego J. Competitive substrate biodegradation during surfactant-enhanced remediation. J Environ Eng 1999; 125:1142-1148.
43. Franzetti A, Di Gennaro P, Bestetti G et al. Selection of surfactants for enhancing diesel-hydrocarbons contaminated media bioremediation. J Hazard Mater 2008; 252:1309-1319.
44. Shreve GS, Inguva S, Gunnan S. Rhamnolipid biosurfactant enhancement of hexadecane biodegradation by Pseudomonas aeruginosa. Mol Mar Biol Biotechnol 1995; 4:331-337.
45. Zhang Y, Miller RM. Enhanced octadecane dispersion and biodegradation by a Pseudomonas rhamnolipid surfactant (biosurfactant). Appl Environ Microbiol 1992; 58:3276-3282.
46. Al-Tahhan RA, Sandrin TR, Bodour AA et al. Rhamnolipid-induced removal of lipopolysaccharide from Pseudomonas aeruginosa: Effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol 2000; 66:3262-3268.
47. Zhong H, Zeng GM, Yuan XZ et al. Adsorption of dirhamnolipid on four microorganisms and the effect on cell surface hydrophobicity. Appl Microbiol Biotechnol 2007; 77:447-455.
48. Chen P, Pickard MA, Gray MR. Surfactant inhibition of bacterial growth on solid anthracene. Biodegradation 2000; 11:341-347.
49. Weber WJ Jr, Huang W, Le Boeuf EJ. Geosorbent organic matter and its relationship to the binding and sequestration of organic contaminants. Colloids Surf A 1999; 151:167-179.
50. Fortin J, Jury WA, Anderson MA. Enhanced removal of trapped nonaqueous phase liquids from saturated soil using surfactant solutions. J Contam Hydrol 1997; 24:247-267.
51. Mulder H, Wassink GR, Breure AM et al. Effect of nonionic surfactants on naphthalene dissolution and biodegradation. Biotechnol Bioeng 1998; 60:397-407.
52. Bai GY, Brusseau ML, Miller RM. Biosurfactant-enhanced removal of residual hydrocarbon from soil. J Contam Hydrol 1997; 25:157-170.
53. Edwards DA, Adeel Z, Luthy RG. Distribution of nonionic surfactant and phenanthrene in a sediment/aqueous system. Environ Sci Technol 1994; 28:1550-1560.
54. Noordman WH, Wachter JH, de Boer GJ et al. The enhancement by surfactants of hexadecane degradation by Pseudomonas aeruginosa varies with substrate availability. J Biotechnol 2002; 94:195-212.
55. Ron EZ, Rosenberg E. Biosurfactants and oil bioremediation. Curr Opin Biotechnol 2002; 13:249-252
56. Lee M, Kang H, Do W. Application of nonionic surfactant-enhanced in situ flushing to a diesel contaminated site. Water Res 2005; 39:139-46.
57. Urum K, Pekdemir T, Gopur M. Optimum conditions for washing of crude oil-contaminated soil with biosurfactant solutions. T I Chem Eng-Lond 2003; 81B:203-209.
58. Harvey S, Elashi I, Val des JJ et al. Enhanced removal of exxon valdez spilled oil from alaskan gravel by a microbial surfactant. Biotechnology 1990; 8:228-230.
59. Van Dyke MI, Couture P, Brauer et al. Pseudomonas aeruginosa UG2 rhamnolipid biosurfactants: structural characterization and their use in removing hydrophobic compounds from soil. Can J Microbiol 1993; 39:1071-1078.
60. Vipulanandan C, Ren X. Enhanced solubility and biodegradation of naphthalene with biosurfactant. J Environ Eng 2000; 126:629-634.
61. Cameotra SS, Bollag JM. Biosurfactant-enhanced bioremediation of polycyclic aromatic hydrocarbons. Crit Rev Environ Sci Technol 2003; 30:111-126.
62. Chu W. Remediation of contaminated soils by surfactant-aided soil washing. Pract Period Hazard Toxic Radioact Waste Manage 2003; 7:19-24.
63. Saichek RE, Reddy KR. Effects of system variables on surfactant enhanced electrokinetic removal of polycyclic aromatic hydrocarbons from clayey soils. Environ Technol 2003; 24:503-515.

64. Miller RM. Biosurfactant-facilitated remediation of metal-contaminated soils. Environ Health Perspect 1995; 103 Suppl 1:59-62.
65. Tan H, Champion JT, Artiola JF et al. Complexation of cadmium by a rhamnolipid biosurfactant. Environ Sci Technol 1994; 28:2402-2406.
66. Ochoa-Loza FJ, Artiola JF, Maier RM. Stability constants for the complexation of various metals with a rhamnolipid biosurfactant. J Environ Qual 2001; 30:479-485.
67. Mulligan CN, Yong RN. The use of biosurfactants in the removal of metals from oil-contaminated soil. (1997) In: Yong RN, ed. Contaminated Ground: Fate of Pollutants and Remediation. London: Thomas Telford Publishers, 1997:461-466.
68. Maslin P, Maier RM. Rhamnolipid-enhanced mineralization of phenanthrene in organic-metal cocontaminated soils. Biorem J 2000; 4:295-308.
69. Gutnick DL, Bach H. Engineering bacterial biopolymers for the biosorption of heavy metals; new products and novel formulations. Appl Microbiol Biotechnol 2000; 54:451-460.
70. Bodour AA, Maier RM. Application of a modified drop- collapse technique for surfactant quantification and screening of biosurfactant-producing microorganisms. J Microbiol Methods 1998; 32:273-280.
71. Chen CY, Baker SC, Darton RC. The application of a high throughput analysis method for the screening of potential biosurfactants from natural sources. J Microbiol Methods 2007; 70:503-510.
72. Torsvik V, Øvreås L, Thingstad TF. Prokaryotic diversity-magnitude, dynamics and controlling factors. Science 2002; 296:1064-1066.
73. Keller M, Zengler K. Tapping into microbial diversity. Nat Rev Microbiol 2004; 2:141-50.